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## PART I - ADMINISTRATIVE

### Section 1. General administrative information

#### Title of project

Identification Of Redband And Rainbow Trout In The N F Clearwater Basin

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**BPA project number:** 20156

**Contract renewal date (mm/yyyy):** ☐ **Multiple actions?**

**Business name of agency, institution or organization requesting funding**

Nez Perce Tribe

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**Business acronym (if appropriate)** NPT

#### Proposal contact person or principal investigator:

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#### NPPC Program Measure Number(s) which this project addresses

This INNOVATIVE NEW PROPOSAL is designed to supplement data collected in the genetic inventory of westslope cutthroat trout (9501600)measure nos 10.3C.4 and 10.3C.5, and will provide important information for loss assessments meas.nos 10.1C.1 and 10.1C.3.

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#### FWS/NMFS Biological Opinion Number(s) which this project addresses

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#### Other planning document references

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#### Short description

We are proposing a 12 month genetic study to identify if native wild redband trout exist in the North Fork Clearwater basin, and determine if introgression from hatchery rainbow trout threatens native redband and cutthroat trout in the basin.

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#### Target species

redband trout, westslope cutthroat trout

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## Section 2. Sorting and evaluation

Subbasin  
Clearwater

### ***Evaluation Process Sort***

CBFWA caucus	Special evaluation process	ISRP project type
Mark one or more caucus	If your project fits either of these processes, mark one or both	Mark one or more categories
<input type="checkbox"/> Anadromous fish <input checked="" type="checkbox"/> Resident fish <input type="checkbox"/> Wildlife	<input type="checkbox"/> Multi-year (milestone-based evaluation) <input type="checkbox"/> Watershed project evaluation	<input type="checkbox"/> Watershed councils/model watersheds <input type="checkbox"/> Information dissemination <input type="checkbox"/> Operation & maintenance <input type="checkbox"/> New construction <input checked="" type="checkbox"/> Research & monitoring <input type="checkbox"/> Implementation & management <input type="checkbox"/> Wildlife habitat acquisitions

## Section 3. Relationships to other Bonneville projects

***Umbrella / sub-proposal relationships.*** List umbrella project first.

Project #	Project title/description

### ***Other dependent or critically-related projects***

Project #	Project title/description	Nature of relationship
9501600	Genetic Inventory of Westslope Cutthroat Trout in the NF Clearwater basin	this proposed project will supplement information that has been acquired in 9501600 testing introgression and species distribution
8740700	Dworshak Rule Curves	this proposed project will determine if a native fish, currently not identified, exists in the NF Clearwater basin and should be considered in the evaluation of multipurpose dam operations on resident fish in Dworshak Reservoir
9709900	Dworshak Impacts Assessment	this proposed project can ultimately be used toward information supporting a consumptive fishery in

		Dworshak Reservoir

## Section 4. Objectives, tasks and schedules

### *Past accomplishments*

Year	Accomplishment	Met biological objectives?

### *Objectives and tasks*

Obj 1,2,3	Objective	Task a,b,c	Task
1	determine if native redband trout exist in the NF Clearwater basin, and identify introgression from hatchery rainbow trout stocked into Dworshak Reservoir	a	Collect tissue samples from 40-50 redband or rainbow trout from 15 sites in the basin
		b	genetically analyze and summarize the results
2	determine if there are trends in the strain of rainbow trout hybridizing with native westslope cutthroat trout in the NF Clearwater basin	a	Collect additional tissue samples from 20-30 trout from 5 sites where westslope cutthroat trout and rainbow trout introgression has been identified in the ongoing genetic inventory study
		b	genetically analyze and summarize the results

### *Objective schedules and costs*

Obj #	Start date mm/yyyy	End date mm/yyyy	Measureable biological objective(s)	Milestone	FY2000 Cost %
1	6/2000	6/2001	determination of the number of sites and individuals with the redband genotype. This information will be related to ensuring viable populations of native trout in the basin,	Annual Report summarizing the findings and relating the results to basinwide distribution data, habitat,	75.00%

			and the possibility of future consumptive fisheries in the basin.	morphological characteristics. Recommendations for resident fish mitigation in the basin	
2	6/2000	6/2001	determination of any identifiable trends in rainbow or redband genotypes in cutthroat X rainbow trout hybrids. This information will be related to the goals of the conservation of viable populations of native trout in the basin		25.00%
				<b>Total</b>	100.00%

#### Schedule constraints

#### Completion date

6/2001

## Section 5. Budget

#### FY99 project budget (BPA obligated):

#### *FY2000 budget by line item*

Item	Note	% of total	FY2000
Personnel	6 mo Project Leader 2 seasonals 2 mo each	%29	32,000
Fringe benefits		%8	8,500
Supplies, materials, non-expendable property	office supplies, vials, racks, ethanol, waders, boots	%2	2,000
Operations & maintenance	4 mo rent, phone, utilities, vehicle	%4	4,500
Capital acquisitions or improvements (e.g. land, buildings, major equip.)		%0	
NEPA costs		%0	
Construction-related		%0	

support			
PIT tags	# of tags:	%0	
Travel	field per diem, travel to AFS conference to present results	%2	2,000
Indirect costs	23%	%10	10,925
Subcontractor	genetic analyses	%46	51,000
Other		%0	
<b>TOTAL BPA FY2000 BUDGET REQUEST</b>			<b>\$110,925</b>

### ***Cost sharing***

<b>Organization</b>	<b>Item or service provided</b>	<b>% total project cost (incl. BPA)</b>	<b>Amount (\$)</b>
		%0	
		%0	
		%0	
		%0	
<b>Total project cost (including BPA portion)</b>			<b>\$110,925</b>

### ***Outyear costs***

	<b>FY2001</b>	<b>FY02</b>	<b>FY03</b>	<b>FY04</b>
<b>Total budget</b>				

## **Section 6. References**

<b>Watershed?</b>	<b>Reference</b>
<input type="checkbox"/>	Benke, R.J. 1992. Native trout of western North America. American Fisheries Society Monograph 6. American Fisheries Society, Bethesda, MD.
<input type="checkbox"/>	Currens, K.P., C.B. Schreck, and H.W. Li. 1990. Allozyme and morphological divergence of rainbow trout above and below waterfalls in the Deschutes River, Oregon. Copeia. 3:730-746.
<input type="checkbox"/>	Dowling, T. E., C. Moritz and J. D. Palmer. 1990. Nucleic acids II: restriction site analysis. In D. M. Hillis and C. Moritz (ed.) Molecular Systematics, Sinauer Associates, Inc., Sunderland.
<input type="checkbox"/>	Felsenstein, J. 1993. PHYLIP: phylogenetic inference package. University of Washington, Seattle.
<input type="checkbox"/>	Fitch, W. M. and M. Margoliash. 1967. Construction of phylogenetic trees. Science 155:279-284.
<input type="checkbox"/>	McElroy, D., P. Moran, E. Bermingham and I. Kornfield. 1991. REAP: the restriction enzyme analysis package. Center for Marine Studies, University of Maine, Orono.
<input type="checkbox"/>	Moffitt, C.M. and T.C. Bjornn. 1984. Fish abundance upstream from

	Dworshak Dam following exclusion of steelhead trout. Technical Completion Report. Project WRIP/371404. Idaho Water and Energy Resources Research Institute. Univ. of Idaho, Moscow, ID.
<input type="checkbox"/>	Morris, D.B., K.R. Richard, and J.M. Wright 1996. Microsatellites from rainbow trout ( <i>Oncorhynchus mykiss</i> ) and their use for genetic study of salmonids. <i>Can. J. Fish. Aquat. Sci.</i> 53:120-126.
<input type="checkbox"/>	Nei, M. 1987. <i>Molecular Evolutionary Genetics</i> . Columbia University Press, New York.
<input type="checkbox"/>	Sambrook, J., E. F. Fritsch and T. Maniatis. 1989. <i>Molecular Cloning: a laboratory manual</i> . Cold Spring Harbor Press, Cold Spring Harbor.
<input type="checkbox"/>	Spruell, P., D.L. Knudsen, J.W. Smithwick, and F.W. Allendorf. 1997. Genetic monitoring of westslope cutthroat trout in the North Fork Clearwater River. Annual Report submitted to the Nez Perce Tribe. Orofino, ID. 15pp.
<input type="checkbox"/>	Swofford, D.L., and R. B. Selander. 1981. BIOSIS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. <i>J. Hered.</i> 72:281-283.
<input type="checkbox"/>	Thurrow, R.F., D.C. Lee, and B.E. Rieman. 1997. Distribution and status of seven native salmonids in the interior Columbia River basin and portions of the Klamath River and Great Basins. <i>North American Journal of Fisheries Management</i> . 17:1094-1110.
<input type="checkbox"/>	Williams, R.N., D.K. Shiozawa, J.E. Carter, and R.F. Leary. 1996. Genetic detection of putative hybridization between native and introduced rainbow trout populations of the upper Snake River. <i>Transactions of the American Fisheries Society</i> . 125:387-401
<input type="checkbox"/>	Williams, R.N., R.F. Leary, and K.P. Currens. 1997. Localized genetic effects of a long-term hatchery stocking program on resident rainbow trout in the Metolius River, Oregon. <i>North American Journal of Fisheries Management</i> . 17:1079-1093.
<input type="checkbox"/>	Wilson, G.M., W.K. Thomas, and A.T. Beckenbach. 1985. Intra- and interspecific mitochondrial DNA sequence divergence in <i>Salmo</i> : rainbow, steelhead, and cutthroat trouts. <i>Canadian Journal of Zoology</i> . 63:288-2094.

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## PART II - NARRATIVE

### Section 7. Abstract

The goal of the resident fish mitigation program is to emphasize the long-term sustainability of viable native fish populations, conserve natural genetic diversity, and ensure that stocking is not interfering with existing native, wild resident fish (1994 FWP secs. 10.1, 10.1A, 10.2A). To obtain this goal, the distribution of native trout within the Columbia basin needs to be defined. Redband trout are identified as a species of concern. Questions pertaining to the presence and behavior of redband trout remain, particularly where their distribution overlaps with steelhead. Populations of rainbow trout exist in the NF Clearwater basin. The source of these fish is unclear considering the extent of

stocking related to fish management and mitigation in the basin. Strains of rainbow trout can be identified using mitochondrial and microsatellite genetic techniques. We will collect samples from 15 tributaries in the drainage where rainbow trout populations have been identified. We will also collect tissue samples from five sites where cutthroat X rainbow trout introgression has been identified. Tissue samples will be collected from 40-50 trout at each site. We will use mitochondrial and microsatellite DNA techniques to study the genetic differentiation within and among rainbow trout populations. Genetic data from this project will be analyzed with several commonly used statistical programs including: NTSYS, PHYLIP, PAUP, SigmaSTAT, DNAsize, and Sigma Scan. Samples from the areas of known introgression will be examined similarly and will lend critical insight into the origin of the rainbow trout strain(s) hybridizing with native cutthroat trout populations. Identifying the strains of rainbow trout will provide valuable information for the management of the species and mitigation in Dworshak Reservoir and the North Fork Clearwater basin.

## **Section 8. Project description**

### **a. Technical and/or scientific background**

Although redband trout have a broad distribution, less is known about their current status than any other salmonid taxa in the Columbia Basin (Thurow et al. 1997). It is generally accepted that redband trout have two co-existing life history types: resident/fluvial, and anadromous (called steelhead). The level of behavioral or genetic segregation between these life history types is largely unknown (Thurow et al. 1997; Currens et al. 1990). However, Wilson et al. (1985) found genetic differences in sympatric rainbow trout and steelhead in coastal streams indicating little hybridization occurred naturally. The Clearwater and Salmon drainages in Idaho are the only subbasins within the Columbia basin where native redband and cutthroat trout are widespread and co-exist (Benke 1992).

The effects of hydropower construction, and subsequent mitigation activities, has not been evaluated in a basin where redband, steelhead, and cutthroat trout populations co-exist. Dworshak Dam, completed in 1972, blocked anadromous runs of salmon and steelhead in the basin, and potentially could have caused the residualization of steelhead, although rainbow/steelhead counts were substantially reduced just after the completion of the dam (Moffitt and Bjornn 1984). In addition to the effects of the construction of Dworshak Dam, the stocking of rainbow trout has been used as mitigation to enhance the fishery in Dworshak Reservoir since 1972, without investigating the impacts on wild populations of trout. The current mitigation activities may be reducing the survival and fitness of a potentially native fishery in the basin. Populations of rainbow trout have been observed in tributaries of the North Fork Clearwater drainage that have morphological characteristics similar to redband trout (D.E. Weigel, NPT, unpublished data). If populations of redband trout persist in the North Fork Clearwater drainage, it is possible that ongoing resident fish mitigation practices could have a detrimental effect on the long-term persistence and genetic integrity of these native populations. Due to widespread stocking of unrelated strains of rainbow trout, the identification of native redband has relied on genetic techniques.

Introgression between native redband populations and hatchery rainbow trout have been documented using genetic methods (Williams et al. 1996; Currens et al. 1990; Williams et al. 1997). Additionally, hybridization between rainbow trout and native westslope cutthroat trout have been detected in the North Fork Clearwater drainage (Spruell et al. 1998). Since redband trout co-evolved with westslope cutthroat trout, we would expect limited hybridization to naturally occur between the two species. Therefore, there may be detectable differences between the strain of rainbow trout hybridizing with the native westslope cutthroat trout within the basin. The genetic method currently being used to detect introgression in the cutthroat trout populations does not provide evidence of rainbow trout strain (Spruell et al. 1997). Other genetic methods need to be coupled to confidently identify the strain of rainbow trout (Williams et al. 1996; Wilson et al. 1985; Currens et al. 1990; Williams et al. 1997). Analysis of mitochondrial DNA restriction fragment length polymorphisms (RFLPs) has revealed differences among rainbow trout and redband trout populations in several instances within the Columbia basin (M. Powell, unpublished data). However, the power of mitochondrial DNA RFLP analysis is limited at this microgeographic scale, and must be utilized in conjunction with other molecular techniques, such as microsatellite DNA, which examine the nuclear genome and are potentially much more polymorphic. RFLP analysis of mitochondrial DNA will be used because of its cost effectiveness and relatively straightforward application. Microsatellite DNA markers will be used to: 1) support and complement the mitochondrial DNA conclusions, and 2) add further precision to our determinations of intraspecific origin and divergence. Microsatellite DNA markers potentially offer a much higher level of resolution regarding genetic variation and differentiation. Using genetic data from mitochondrial and microsatellite DNA will strengthen the results of each and reduces potential bias in conclusions drawn from using only one data set.

#### **b. Rationale and significance to Regional Programs**

We are proposing an innovative study identifying native redband trout populations in the North Fork Clearwater basin currently not documented, and evaluating the effects of hydropower and subsequent mitigation stocking activities on redband and westslope cutthroat trout and their interactions. The overall goal of the FWP is to support and rebuild populations of native species in native habitats, while protecting and enhancing habitat that is irrevocably changed (Sections 2, 2.1, 2.2). The FWP identifies redband trout as a species of concern, and stresses the goal of preserving natural genetic diversity within native, wild populations of resident fish (Section 10). Redband trout populations are documented to co-exist with steelhead in the Clearwater basin, Idaho (Thurrow et al. 1997). The construction of Dworshak Dam blocked the anadromous fish runs native to the NF Clearwater basin. The adaptation of redband or steelhead populations within a drainage blocked by hydropower has also been undocumented. In addition to the effects of the construction of Dworshak Dam, the stocking of rainbow trout has been used as mitigation to enhance the fishery in Dworshak Reservoir since 1972, without investigating the impacts on wild populations of trout. The current mitigation activities may be reducing the survival and fitness of a potentially native fishery in the basin. The FWP requests the evaluation of detrimental effects of artificial



propagation on the long-term sustainability of native species, and calls for a thorough comprehensive approach to conserving genetic diversity in native species when stocking is proposed. Interbreeding is specifically identified as a concern, and the plan stresses minimizing the impacts of hatchery fish on wild fish (Section 10.2.A).

This proposed innovative project meets the following resident fish high priority criteria: 1) this project protects the health of existing resident fish populations; 2) if a native redband trout population is identified in the North Fork Clearwater basin, it could support an important fishery; and 3) this project may address losses to resident fish populations caused by hydropower construction and subsequent resident fish mitigation stocking activities. This project will directly supplement data collected under the genetic inventory of westslope cutthroat trout (project no. 9501600). Measure number 10.3C.4 authorizes the NPT to investigate introgression in the westslope cutthroat trout in the NF Clearwater basin, and 10.3C.5 authorizes BPA to fund the project. Additionally, this project will provide important information that should be included in loss assessments authorized and funded under measure numbers 10.1C.1 and 10.1C.3.

Our project also contributes information toward achieving the goals identified in the proposed draft MYIP. The CBFWA DAIWP (MYIP section 6.6.5.3A) addresses two resident fish objectives: 1) To maintain and restore population productivity reduced by hydropower development and operations to healthy levels which provide opportunities for consumptive and nonconsumptive native fisheries; and 2) To ensure population levels of native fish above minimum viable population sizes which maintain adaptability and genetic diversity, and maximize probability of survival. The ISRP identified a need for baseline inventory information on native resident fish stocks in the Columbia basin (ISRP 1998 review of FWP program and projects p. 16). Resident fish mitigation has been ongoing in the North Fork Clearwater basin for more than 25 years without defining baseline life history and population interactions. Additionally, IDFG has proposed introducing hatchery surplus steelhead into the NF Clearwater drainage. The rationale for the proposed activity is to provide nutrients to the basin, and fill a niche vacated by the lost anadromous runs of steelhead. It is possible native redband populations have filled this “vacant niche.” However, mitigation cannot be adequately addressed unless we define existing resident fish populations in the basin. The goals of the FWP cannot be met if this baseline information is not acquired. The results of this proposed innovative research project will be used to identify native species in the basin, minimize the impacts on existing native wild populations of westslope cutthroat trout, and maintain pure genetic composition, and viable populations of all native trout in the basin.

### **c. Relationships to other projects**

This project is related to other Dworshak Reservoir mitigation projects (project numbers 9501600, 8740700, and 8709900) implementing resident fish mitigation that provides a consumptive fishery ( $>0.5$  fish/hr), but does not conflict with the preservation of other native wild fish, and minimizes the impacts of hatchery trout on wild trout. These projects are involved in the Dworshak resident fish mitigation review, and the System Operation Review, and coordinate with other agencies (CoE, USFWS, IDFG) on the management of fisheries mitigation in the basin.

Little is known about the status of redband trout where the distribution overlaps with steelhead. Determining the existence of native trout in the North Fork Clearwater basin is important to conserving native trout in native habitats. Introgression between hatchery rainbow trout and native redband trout is a concern. Furthermore, this study will provide additional information on the degree of genetic introgression by several different strains of rainbow trout. The information gained from this project will provide important genetic data related to the adaptations of rainbow and redband trout to basins altered by hydropower.

**d. Project history** (for ongoing projects)

This is a new project being proposed for innovative new project funds.

**e. Proposal objectives**

Objective 1. Determine if redband trout exist in the North Fork Clearwater basin, and whether introgression has occurred between native redband (if detected), and hatchery rainbow trout.

Null Hypothesis: Redband trout do not exist in the North Fork Clearwater basin. Hence, all rainbow trout are identifiable as originating from hatchery strains introduced into Dworshak Reservoir.

Assumptions: Collection sites represent all presently identified areas where rainbow trout have been observed. The discrimination of hatchery origin rainbow trout from native redband trout is statistically robust within the samples collected from each location.

Measureable Objectives: Assessing the status of native redband trout contributes information to the measureable biological objective of maintaining viable populations of native fish (as measured as 150-300 breeding individuals with a 95% probability of persistence). Identifying whether a population exists is the first strategy in determining the status.

Possible Results:

1. All rainbow trout sampled from the basin match haplotypes of hatchery rainbow trout introduced into Dworshak Reservoir (predominantly Arlee, Shasta, and Hayspur rainbow trout).
2. All rainbow trout sampled from the basin match haplotypes of inland redband trout, indicating a population exists in the basin.
3. Rainbow trout sampled from the basin match haplotypes of hatchery rainbow and inland redband trout, but do not show introgression.
4. Rainbow trout sampled from the basin match haplotypes of hatchery rainbow and inland redband trout, and do show introgression in some populations.

Objective 2. Determine if there are any trends in the rainbow trout haplotypes detected in introgressed populations of cutthroat and rainbow trout.

Null Hypothesis: Introgression in westslope cutthroat trout populations results from random matings with rainbow trout, indicated as no trends in rainbow trout markers detected in introgressed populations.

Assumptions: The discrimination of hatchery rainbow trout and/or native redband trout from native cutthroat trout is statistically robust, and the samples represent a random subset of individuals from each location.

Measurable Objectives: Information gained from determining the impact of rainbow trout on native westslope cutthroat trout populations contributes information to the measurable biological objective of maintaining viable populations of native fish (as measured as 150-300 breeding individuals with a 95% probability of persistence).

Expected Results:

1. All rainbow trout markers detected in introgressed populations of cutthroat and rainbow trout are from hatchery rainbow trout.
2. All rainbow trout markers detected in introgressed populations of cutthroat and rainbow trout are from inland redband trout.
3. Rainbow trout markers detected in introgressed populations of cutthroat and rainbow trout are from both inland and hatchery rainbow trout.

## **f. Methods**

Objective 1. Determine if redband trout exist in the North Fork Clearwater basin, and whether introgression has occurred between native redband (if detected), and hatchery rainbow trout.

Objective 2. Determine if there are any trends in the rainbow trout haplotypes detected in introgressed populations of cutthroat and rainbow trout.

The genetic inventory of westslope cutthroat trout project (9501600) has identified streams where rainbow trout and introgressed cutthroat X rainbow trout populations exist in the basin. This study will supplement tissue samples collected by the westslope cutthroat trout project to further define rainbow trout populations in the basin. There are no known risks to cutthroat or rainbow trout associated with tissue sampling as fin clips. RFLP analysis of mitochondrial DNA will be used because of its cost effectiveness and relatively straightforward application. Microsatellite DNA markers will be used to: 1) support and complement the mitochondrial DNA conclusions, and 2) add further precision to our determinations of intraspecific origin and divergence. Microsatellite DNA markers potentially offer a much higher level of resolution regarding genetic variation and differentiation. Using genetic data from mitochondrial and microsatellite DNA will strengthen the results of each and reduces potential bias in conclusions drawn from using only one data set.

Objectives 1 and 2:

Collection and Morphometrics

Rainbow trout will be collected from 15 tributaries in the North Fork Clearwater basin where rainbow trout populations have been identified. Cutthroat X rainbow trout hybrids will be collected from 5 sites where introgression has been detected. Tissue samples will be collected from 40-50 trout at each site. Trout will be collected with a backpack electroshocker. Tissue samples will be taken as small fin clips from the pelvic or caudal fins, and preserved in 95% ethanol. Morphometric characteristics will be

collected for each fish from which tissue is collected, and include both continuous and categorical variables. Morphometric characteristics will include: body measurements, slash intensity, spot pattern, fin spotting, and presence/absence of hyoid teeth. The location of each sampling site will be marked on a topographic map.

### Genetic Methods

#### Mitochondrial DNA Analyses

We will employ the polymerase chain reaction (PCR) to amplify four separate gene regions of mitochondrial DNA (mtDNA): Cytochrome b, NADH dehydrogenase subunit 1, NADH dehydrogenase subunit 2, and NADH dehydrogenase subunits 5/6. These sequences of DNA code for proteins active in the oxidative-phosphorylation pathway, carried out within the mitochondrial matrix. The DNA found in mitochondria is circular in conformation, small (approximately 16,000 base pairs in size), maternally inherited, and non-recombinatory. Mitochondrial DNA exhibits a relatively rapid rate of nucleotide change or evolution (when compared to nuclear gene sequences). For these reasons, mtDNA has been widely applied in fisheries population studies. Tissue samples from each *O. mykiss* or putative *O. clarki* hybrid will be stored separately in 70% ethanol or lysis buffer (50 mM Tris-HCl, pH 8.0, 200 mM NaCl, 50 mM EDTA, 1% sodium dodecyl sulfate, 0.2% dithiothreitol) until DNA is extracted using methods modified from Sambrook et al. (1989) and Dowling et al. (1990). The polymerase chain reaction (PCR) will be used to amplify mtDNA sequences from each sample using nucleotide primers specific for the mitochondrial Cytochrome b and NADH dehydrogenase subunit 1, 2, and 5/6 gene regions (LGL Ecological Genetics). Amplified mtDNA gene regions are digested using 13 Type II restriction endonucleases. The resulting mtDNA fragments are separated by electrophoresis using agarose or polyacrylamide gels. Gels are stained with ethidium bromide and restriction fragment patterns visualized using UV light. Photographs of each gel are converted into computer image files using a ScanMan scanner and ScanMan 2.0 software (Logitech). Restriction fragment length polymorphisms (RFLPs) observed among samples will be measured using SigmaScan Pro 2.0 (Jandel Scientific 1995), then given alphabetical designations as simple haplotypes. Fragment sizes of each RFLP from each gene region will be estimated by comparison to a size standard, pUC-19 marker (Bio-Synthesis). Alphabetical designations from RFLPs of each mitochondrial gene region will be combined into composite haplotypes. An estimate of the number of nucleotide substitutions per site (p) for each RFLP is calculated via the Nei (1987) method using REAP 4.0 (Restriction Enzyme Analysis Package) (McElroy et al., 1991) then used to generate a matrix comparing p values (distance) between all pairs of identified composite haplotypes. The KITSCH program in PHYLIP 3.5 (Felsenstein 1993) which assumes independence and equal rates of evolutionary divergence is used to generate a distance dendrogram via the least-squares method of Fitch and Margoliash (1967) to illustrate the estimated evolutionary relationships and distance among the identified composite rainbow/redband/cutthroat haplotypes.

#### Nuclear DNA Analyses

This project will examine several types of nuclear primers used to PCR amplify microsatellite sequences of variable in size and sequence among and within the collected populations of rainbow trout. The methods of DNA isolation are the same as mtDNA

analyses. This method is also potentially more cost effective than some other nuclear DNA methods and provides a greater probability of detecting polymorphisms with limited sample sizes. Currently, two microsatellite primer sets in particular are being investigated at the University of Idaho for their utility in discriminating between redband and rainbow trout. The primers were developed at the Marine Gene Probe Laboratory at Dalhousie University. The general characteristics and utility of these *O. mykiss* microsatellite primers originally named PuPuPy and Omy 77 have been published (Morris et al. 1996). These two primers have been modified by the University of Idaho and currently show a fair degree of variation (<20 alleles) making them appropriate for population level work. Alternatively, several other microsatellite primer sequences are available from numerous sources which can be utilized if the data generated from the currently selected primers are inconclusive for testing the hypotheses addressed in this project.

Both sets of microsatellite primers are combined in the same reaction sample for the determination of fragment sizes (referred to as multiplexing), the PCR amplified sequences are analyzed using an automated DNA fragment analyzer (310 Genetic Analyzer, ABI/Perkin-Elmer). The analysis of the resulting PCR fragments from each individual can be compared. Computer algorithms that score and compile allele frequency data such as BIOSIS-1 (Swofford and Selander 1981) and GenePop will be used to compare microsatellite diversity among and within sample locations and determine the extent of introgression.

#### Data Analysis

We will test for differences in haplotypes within and between rainbow trout collected at 15 sites in the NF Clearwater drainage. We will also test for differences within and between rainbow trout haplotypes detected in cutthroat X rainbow trout hybrids from five sites in the NF Clearwater basin. Genetic data from this project will be analyzed with several commonly used statistical programs including: NTSYS, PHYLIP, PAUP, SigmaSTAT, DNAsize, and Sigma Scan. Sites will be mapped in GIS to indicate the distributions and sample sites of the different strain(s) of rainbow trout. Morphometric characteristics will be analyzed with the genetic identification of the trout. The data will be analyzed using ANOVAs, MANOVAs, logistic regression, and/or discriminant function analysis. The different statistical analyses will be evaluated for the data meeting the necessary assumptions. Similar analyses will have been completed for the westslope cutthroat trout genetic introgression project (9501600), and we expect to handle the data with the most suited analyses identified by this project. Furthermore, distribution and habitat associated with the different species of trout will also be described by the westslope cutthroat trout project.

#### **g. Facilities and equipment**

The NPT will provide office space, storage, and shared office equipment (fax, photocopier, internet access, etc) for the project in the Orofino Field Office. The location of the Field Office reduces travel time to the field sites. The project will access

computers and equipment already purchased for project 9501600 (Genetic inventory of westslope cutthroat trout in the North Fork Clearwater basin), and will contribute funds to a shared vehicle and expendable office supplies. The westslope project has a 4-wheeler, trailer, backpack electroshocker, and two computers.

The Aquaculture Research Institute (ARI) at the University of Idaho maintains a fisheries genetics laboratory on the main campus in Moscow, Idaho and a second genetics facility at the Center for Salmonid and Freshwater Species at Risk. These laboratories are directed by a full time research scientist (Dr. M. Powell) and have a current staff of eight including, a doctoral research assistant, several full time and part time lab technicians, and students in addition to affiliate faculty with other collaborative projects. Both laboratories contain all the equipment necessary to collect, generate, and analyze molecular genetic data necessary for the proposed project. This includes all laboratory equipment, data analysis software, office, and clerical support. Genetic analyses will be divided between the two facilities to expedite the completion of this project. The majority of the nuclear DNA analysis will be conducted at the Center for Salmonid and Freshwater Species at Risk using the automated DNA fragment analyzer at this location. The mitochondrial DNA analyses will be performed at the ARI genetics facility in Moscow. The fisheries genetics laboratories of the University of Idaho's Aquaculture Research Institute, provide a central clearinghouse for molecular systematics and comprehensive genetic evaluation in fisheries. The Laboratories' mission is to establish necessary and critical population genetic data for the benefit of all managers, agencies, and tribes.

#### **h. Budget**

This project has been designed to answer important questions about the source of rainbow trout populations in the NF Clearwater basin at minimal cost. This project will benefit from information and analyses designed by the westslope cutthroat trout study. The NPT personnel costs include a project leader (for 6 months) who will be involved in sample collection, site selection, analysis, and interpretation of the results. The NPT will also have 2 seasonal bio-aides for data collection in the backcountry. The U of I will analyze the genetic tissue samples under subcontract with the NPT. The genetic analysis is the bulk of the work for this project, and comprises 47% of the budget. Dr. Madison Powell will oversee the analyses and interpretation of the genetic results, at no cost to the project. The subcontract cost includes: 1 full-time scientific aide, 1 part time senior aide, supplies, O&M, and indirect. The other line items under the proposed budget include supplies for collecting and storing tissue samples, and miscellaneous office supplies. The NPT is billing O&M (rent, phone, utilities) for 6 months of the 12 months project, and will rent a 4X4 vehicle for 3 months to access sites. The project will share equipment purchased for the westslope cutthroat trout project. The westslope project has 2 computers, a 4-wheeler, trailer, and a backpack electroshocker.

### **Section 9. Key personnel**

This project is a 12 month project, and will utilize 2 principal investigators as key personnel. The NPT Principal Investigator will only bill the project for 6 months. The U

of I Principal Investigator will not bill the project. Seasonal personnel will be hired upon receipt of the funding, and technical personnel for genetic analyses are included in the subcontract genetic analysis line item.

Dana Weigel, Project Leader, Nez Perce Tribe Fisheries, Orofino Field Office, 3404 Hwy 12, Orofino, ID 83544

#### EDUCATION

M.S. Fisheries, University of Minnesota 1994

B.S. Aquatic Environments, Allegheny College 1991

#### RESEARCH EXPERIENCE AND PUBLICATIONS

Project Leader, Nez Perce Tribe, Orofino, ID, Sept 1996 – present.

Project: Genetic inventory of westslope cutthroat trout in the North Fork Clearwater basin.

Reports: **Weigel, D.E.** 1997. The genetic inventory of westslope cutthroat trout in the NF Clearwater basin, Idaho. Annual Report prepared for the Bonneville Power Administration. Contract No. 95BI61768, Project No. 9501600. 13pp.

**Weigel, D.E.** and S. Cross. 1998. The genetic inventory of westslope cutthroat trout in the NF Clearwater basin, Idaho. Annual report prepared for the Bonneville Power Administration. Contract No. 97AM30423, Project No. 9501600.

Research Assistant, University of Minnesota, Department of Fisheries and Wildlife, St. Paul MN, Sept 1991 – March 1994

Thesis Title: **Weigel, D.E.** and P.W. Sorensen. Longitudinal distribution of brook, brown, and rainbow trout in a midwestern stream cannot be explained by habitat variables, submitted Transactions of American Fisheries Society 1997.

Co-Author: Sorensen, P.W., T.E. Essington, J. Cardwell, and **D.E. Weigel**. 1995. Hybridization and spawning behavior of brook and brown trout in a small stream. Canadian Journal of Fisheries and Aquatic Sciences. 52:1958-1965.

#### TECHNICAL EXPERIENCE

Fisheries Biologist, Clearwater Biostudies Inc., Canby, OR, June –Sept 1996.

Project: Steam surveys under contract with the USFS Clearwater and Nez Perce National Forests

Fisheries Biologist, University of Idaho, Cooperative Fisheries Research Unit, Moscow, ID, April – June 1996.

Project: Radiotelemetry of adult chinook salmon at Ice Harbor Dam

Fisheries Biologist, M&M Environmental Enterprises, Boise, ID, June-Dec 1995.

Project: Stream surveys under contract with the USFS Payette National Forest

Fisheries Consultant, Vermont Natural Resource Council, Montpelier, VT, April – June 1995

Project: Prepare expert testimony evaluating FERC dam relicensing regulation, and evaluate the flow regulation studies and proposed fish passage facilities

Fisheries Biologist, USFS Intermountain Research Station, Boise, ID, Aug –Oct 1994.

Project: Monitoring the movements and genetic exchange of resident and migratory bull trout

Fisheries Biologist, National Biological Survey, Cook WA, April – July 1994

Project: Monitoring the movement of chinook and steelhead smolts through reservoirs and dams on the Snake and Columbia Rivers using radiotelemetry and hydroacoustics.

Research Assistant, Rocky Mountain Biological Lab, Gothic, CO, June-Sept 1991.

Project: Evaluating the costs and benefits of paedomorphosis versus metamorphosis in tiger salamanders and identifying the species composition of invertebrates in high elevation ponds.

#### TRAINING

University of Idaho, Applications of Multivariate Statistical Methods 1997.

USFWS, Fish Genetics, 1997.

#### JOB DUTIES

Literature reviews, report writing, experimental design, data analysis, computer modeling, speaking to peer and local interest groups, budget planning and management, writing proposals, administer contract and subcontract, personnel management, planning logistics, provide scientific advice to the agency, coordinate activities with other agencies and projects

## MADISON S. POWELL

### Education:

Ph.D., 1995, Texas Tech University

M.S., 1990, University of Idaho

B.S., 1985, University of Idaho

**Current employer:** University of Idaho, Hagerman Fish Culture Experiment Station  
3059 F National Fish Hatchery Road, Hagerman, ID 83332, (208) 837-9096  
FAX: (208) 837-6047, email fishdna@micron.net

**Current Responsibilities:** Research scientist; supervise fisheries genetics laboratories and lab personnel at the Aquaculture Research Institute and the Hagerman Fish Culture Experiment Station.

### Previous employment:

1997-present	Research Scientist, Hagerman Fish Culture Experiment Station, University of Idaho, Hagerman, Idaho
1996-1997	Research Scientist, Aquaculture Research Institute, University of Idaho, Moscow, Idaho
1995-1996	Postdoctoral Fellow, Aquaculture Research Institute, University of Idaho, Moscow, Idaho
1995	Ph.D., Zoology, Texas Tech University
1990	M.S., Zoology, University of Idaho
1985	B.S., Zoology/Biology, University of Idaho

### Technical experience:

DNA and RNA isolation, molecular cloning, genomic libraries, DNA fingerprinting, automated sequencing, PCR amplification, RFLP analysis, RAPD analysis, *in vitro* transcription, fluorescence *in situ* hybridization, karyotyping, cell and tissue culture, nucleotide and protein electrophoresis, liquid chromatography, HPLC analysis, small animal surgery, field collection, and identification.

### Five publication closely related to this project:

- Williams, R.N., **M.S. Powell**, R.P. Evans, and D.K. Shiozawa. 1998. Genetic Analysis of Putative Yellowstone Cutthroat Trout samples from the Henry's Fork Subbasin. Center for Salmonid and Freshwater Species at Risk, University of Idaho. Technical Report. Pp 1-9.
- Powell, M.S.** V.L. Paragamian, and J.C. Faler. 1988. Genetic characteristics of burbot in the Kootenai River drainage of Montana, Idaho, and British Columbia. Proceedings of the International Congress on the Biology of Fish. Burbot Symposium. Pp. 1-4.
- Anders, P. and **M.S. Powell**. 1998. Comprehensive management and conservation of Columbia Basin white sturgeon (*Acipenser transmontanus*): A zoogeographic approach. Proceedings of Ecosystem Based Management in the Upper Columbia River Basin. Pp. 53-54.
- Paragamian, V.L., **M.S. Powell**, J.C. Faler, and S. Snelson. (accepted for publication) Mitochondrial DNA analysis of burbot *Lota lota* stocks in the Kootenai River Basin of British Columbia, Montana, and Idaho. *Trans. Amer. Fish. Soc.*
- Powell, M.S.** and J.C. Faler. Genetic differentiation among early and late spawning populations of kokanee salmon. In preparation, *Can. J. Fish and Aquat. Sci.*



## **Section 10. Information/technology transfer**

Determining the genotypes and phenotypes of populations of redband trout has not been done in a basin where populations of steelhead, redband, and cutthroat trout co-exist. Therefore, the results of the project will be a significant contribution to the definition of redband trout in the Columbia basin, and will be utilized by all fish managers. Information from the project will be distributed by Annual Reports submitted to BPA under the terms of our contract. Additionally, we plan to incorporate professional society meetings (such as the Idaho and Oregon Chapters of AFS), and inter-agency information sharing (particularly with the CoE, USFWS, and IDFG). We anticipate the results from this project will be publishable in a peer-reviewed journal, and will provide important management information to agencies throughout the Columbia basin. Furthermore, the information gained from this project will be used by the CoE for resident fish mitigation planning in Dworshak Reservoir, and by the USFWS in their status review of redband trout as a candidate for protection under the ESA.

**Congratulations!**